Research Article

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A four-component synthetic attractant for *Drosophila suzukii* (Diptera: Drosophilidae) isolated from fermented bait headspace

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Abstract

BACKGROUND: A mixture of wine and vinegar is more attractive than wine or vinegar to spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), and ethanol and acetic acid are considered key to that attractiveness. In addition to ethanol and acetic acid, 13 other wine and vinegar volatiles are antennally active to *D. suzukii* and might be involved in food finding.

RESULTS: Out of the 13 antennally active chemicals, acetoin, ethyl lactate and methionol increased fly response to a mixture of acetic acid and ethanol in field trapping experiments. A five-component blend of acetic acid, ethanol, acetoin, ethyl lactate and methionol was as attractive as the starting mixture of wine and vinegar in field tests conducted in the states of Oregon and Mississippi. Subtracting ethyl lactate from the five-component blend did not reduce the captures of flies in the trap. However, subtracting any other compound from the blend significantly reduced the numbers of flies captured.

CONCLUSION: These results indicate that acetic acid, ethanol, acetoin and methionol are key olfactory cues for *D. suzukii* when attracted to wine and vinegar, which may be food-finding behavior leading flies to fermenting fruit in nature. It is anticipated that this four-component blend can be used as a highly attractive chemical lure for detection and management of *D. suzukii*. Published 2013. This article is a U.S. Government work and is in the public domain in the USA.

Keywords: *Drosophila suzukii*; spotted wing drosophila; feeding attractant; trap; lure

1 INTRODUCTION

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), native to south-eastern Asia, is a newly emerging polyphagous invasive pest in North America and Europe. Unlike other closely related *Drosophila* species that infest mainly damaged and overripening fruits and thus are not considered serious pests,[1] *D. suzukii* can break the skin of maturing and undamaged healthy fruits and oviposit into them using its serrated ovipositor, making it of great concern as a pest of maturing and ripening fruits.[2–7] The crop damage and potential economic loss caused by *D. suzukii* to fruit growers has been estimated at $US 511 million year^{-1} for small fruit growers in three western US states (California, Oregon and Washington) if damage due to *D. suzukii* were to reach 20% crop loss in cherries, blueberries, strawberries and caneberries in each state,[6] and at €3 million year^{-1} just for small fruit growers in Trento Province in northern Italy,[8] with benefits to *D. suzukii* management estimated to outweigh the costs of monitoring and management of *D. suzukii* and potential revenue losses due to the infestation.[9]

Baits made from fermented food materials are used in traps to detect and monitor *D. suzukii*.[10–12] These baits, such as apple cider vinegar, have had drawbacks such as lack of detection of fly activity before crop damage occurs, difficulty in sorting and identifying *D. suzukii* captured because of a strong response by non-target insects, difficulties in handling and dispensing of the baits and potential variance in bait attractiveness. A synthetic chemical lure in an appropriate trap could provide growers with an improved means to monitor crops to determine the presence and changes in populations of *D. suzukii*, potentially reducing the number of insecticide sprays when the fly is not present, and improving crop protection by providing adequate warning of risk to the crop. A mixture of wine and vinegar was more attractive to *D. suzukii* in the field than either wine or vinegar, and a chemical lure based on that combination of materials may be most useful for monitoring of *D. suzukii*.[11] Acetic acid and ethanol are key to *D. suzukii* attraction to these materials, but do not account for much of the fly response to wine and vinegar.[12] Recently, 13 volatile chemicals from wine and vinegar headspace, in addition to acetic acid and ethanol, were determined to be detected by *D. suzukii*, using a combination of gas chromatography–electroantennographic detection (GC-EAD) and gas chromatography–mass spectrometry.[13] In that study, two

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synthetic chemical blends composed of acetic acid, ethanol and all 13 of the other wine or vinegar EAD-active chemicals were attractive, but were significantly less attractive than the starting material – the mixture of wine plus vinegar. A laboratory bioassay was then used to determine potentially antagonistic compounds in the original blends of EAD-active compounds. Blends of the EAD-active wine compounds and EAD-active vinegar compounds minus compounds found to be antagonistic in the laboratory assays were not statistically different from the wine plus vinegar, but were still 19 and 42% lower respectively in their attractiveness to *D. suzukii*. These results were not conclusive, and yet suggest some room for improvement to the attractiveness of the chemical lure.[13]

It is known that laboratory assays with semiochemicals can provide results that differ from field experiments.[14] Patterns of *D. suzukii* catches in traps in the field may differ from laboratory test results owing to multiple biotic and abiotic factors that differ between the laboratory and field situations, as well as differences in the behavioral responses that are measured. The authors report here on three field studies that further evaluated *D. suzukii* responses to EAD-active wine and vinegar compounds. The first of these field studies, referred to as the 'add-on' study, determined which of the 13 EAD-active wine and vinegar volatile chemicals were attractive to *D. suzukii* when presented individually, and which of the 13 chemicals improved *D. suzukii* attraction to the mixture of acetic acid and ethanol (i.e. were coattractive). The second study evaluated *D. suzukii* attraction to a chemical blend that combined chemicals that were attractive or coattractive with acetic acid and ethanol in the preceding add-on study. The third study, referred to as the 'drop-out' study, determined which of the coattractants identified in the first of these field experiments were required for maximum attractiveness of a chemical blend to *D. suzukii*.

2 EXPERIMENTAL

2.1 Trap design

Experiments were conducted in Marion County, Oregon, in areas of abundant wild blackberry (*Rubus*) species and commercial blueberry crops, and in Stone County, Mississippi, in areas of pine land and both commercial and research plots of blueberry. The Dome trap (Trappitt trap; Agrisense Ltd, Pontypridd, UK) was used for field trapping, as described.[12] In brief, the trap is yellow on the bottom third and clear on the top two-thirds, with a 5 cm diameter bottom entry hole for attracted insects. The trap bottom holds 300 mL of drowning solution. Various aqueous solutions (water, 7.2% ethanol + 1.6% acetic acid in water, 60% wine + 40% vinegar, 7.2% ethanol + 1.6% acetic acid + 2% ethyl acetate in water) were used as the trap drowning solution,[13] depending on the treatment (see below). All of the drowning solutions contained 0.0125% of unscented dishwashing detergent (Palmolive Clear and Clean Spring Fresh dishwashing soap; Colgate-Palmolive Company, New York, NY) to reduce the surface tension and enhance retention of *D. suzukii* in traps, and 1% boric acid (Fisher Scientific, Santa Clara, CA) to inhibit microbial growth. Synthetic chemicals other than acetic acid, ethanol and ethyl acetate were dispensed from vials suspended in the top center of the inside of traps by wire. For each field experiment, a randomized complete block design was used with ten replications. Traps were placed at a height of 1 m, and were 20 m apart. Insects were collected weekly. Traps and drowning solutions were replaced weekly. Vials containing synthetic chemicals were not replaced during the 2 week trapping period.

2.2 Synthetic chemical lures

In the testing of synthetic chemicals in the field, ethanol, acetic acid and ethyl acetate were added to the aqueous drowning solution of traps owing to their solubility in water and high release rates from wine and vinegar, and all other compounds were dispensed from 4 mL polypropylene vials (Nalgene Nunc International, Rochester, NY), as described previously.[13] Chemicals were released from vials by diffusion through a 3 mm diameter hole in the vial lid. Depending on treatments and experiments, neat synthetic chemicals were loaded onto cotton in vials singly or as a mixture, except for acetoin. Acetoin is a solid at room temperature and was not miscible with other compounds. Therefore, it was dissolved in water 1:1 (w/w) at 70 °C for loading into vials. For mixtures, the compounds were added at the ratios released from wine or vinegar.[13] For single compounds, 1 mL of neat material was loaded into the vial, although a 2 mL solution of acetoin in water was used when acetoin was tested.

2.3 Chemicals

Ethyl butyrate (99%, CAS No. 105-54-4, vapor pressure 12.80 mmHg at 25 °C), 3-hydroxybutan-2-one (acetoin) (≥96%, CAS No. 513-86-0, vapor pressure 2.69 mmHg at 25 °C), 3-methylbutyl acetate (isoamyl acetate) (98%, CAS No. 123-92-2, vapor pressure 5.60 mmHg at 25 °C), 2-methylbutyl acetate (99%, CAS No. 624-41-9, vapor pressure 7.85 mmHg at 25 °C), 3-methylsulfanylpropan-1-ol (methionol) (≥98%, CAS No. 505-10-2, vapor pressure 0.16 mmHg at 25 °C), ethyl (2*E*,4*E*)-hexa-2,4-dienoate (ethyl sorbate) (≥97%, CAS No. 2396-84-1, vapor pressure 0.42 mmHg at 25 °C) and 2-phenylethanol (≥99%, CAS No. 60-12-8, vapor pressure 0.07 mmHg at 25 °C) were purchased from Sigma-Aldrich (St Louis, MO). Ethyl 2-hydroxypropionate (ethyl lactate) (>97%, mixture of enantiomers, CAS No. 97-64-3, vapor pressure 1.16 mmHg at 25 °C), 3-methylbutyl 2-hydroxypropionate (isoamyl lactate) (>98%, CAS No. 19329-89-6, vapor pressure 0.07 mmHg at 25 °C) and diethyl butanedioate (diethyl succinate) (>99%, CAS No. 123-25-1, vapor pressure 0.44 mmHg at 25 °C) were purchased from TCI America (Portland, OR). Ethyl 3-hydroxybutyrate (grape butyrate) (99%, CAS No. 5405-41-4, vapor pressure 0.36 mmHg at 25 °C) was purchased from Arcos Organics (Morris Plains, NJ). Ethanol (200 proof, CAS No. 64-17-5, vapor pressure 44.60 mmHg at 20 °C), acetic acid (99.8%, CAS No. 64-19-7, vapor pressure 11.40 mmHg at 20 °C), ethyl acetate (99.9%, CAS No. 141-78-6, vapor pressure 73 mmHg at 20 °C) and 1-hexanol (>95%, CAS No. 111-27-3, vapor pressure 0.95 mmHg at 25 °C) were purchased from Pharmco (Brookfield, CT), Fisher Scientific (Pittsburgh, PA), EM Science (Gibbstown, NJ) and J. T. Baker (Philipsburg, NJ) respectively.

2.4 Add-on study

This trapping study had two objectives: (1) to determine whether any of the 13 EAD-active chemicals individually could attract *D. suzukii*; (2) to determine whether any of the same chemicals could improve *D. suzukii* attraction to the mixture of acetic acid and ethanol. The 13 compounds were tested in seven discrete tests, with one or two compounds evaluated in each test (Table 1). Details of treatments are shown in Table 1. Five of these seven tests were conducted from 23 February to 5 May 2012 in Marion County, Oregon. By June 2012, the *D. suzukii* populations at the Oregon site were too low to continue the testing. The remaining two tests of this experiment were conducted from 11 June to 20 July 2012 in Stone County, Mississippi. As the *D. suzukii* population level varied during the trapping period, a significant difference was used between the blank traps and the traps baited with a mixture of acetic acid and ethanol as a qualifier to determine whether a particular add-on experiment was a valid test.[12] There were no ripe blackberry or blueberry fruits at the Oregon trap sites during this study, but, at the Mississippi sites, mature blueberries were present in June and July.

2.5 New synthetic blend study

The objectives of this second study were: (1) to determine the attractiveness of a new chemical blend relative to the original starting material (mixture of wine plus vinegar); (2) to assess the new blend in comparison with the previous chemical blends[13] which were based on EAD and laboratory assay results; (3) to confirm the relative attractiveness of the new blend in the two geographically distant locations. This study was conducted from 24 July to 7 August 2012 in Stone County, Mississippi, and from 22 August to 5 September 2012 in Marion County, Oregon, as two distinct tests. At the Mississippi sites, commercial blueberries had been harvested, but ripe berries were still abundant. At the Oregon sites, ripe wild blackberries and ripe commercial blueberries were abundant during this time period.

In both tests, four trap-bait treatments were compared: (1) a mixture of wine and vinegar as a positive control or standard; (2) the wine chemical blend[13] (W2 blend); (3) the vinegar chemical blend[13] (V2 blend); (4) a new chemical blend. All drowning solutions contained boric acid and soap. For treatment 1, a mixture of 60% Merlot wine (12% ethanol; Carlo Rossi Reserve Merlot, Modesto, CA) and 40% rice vinegar (4% acidity; Safeway Select Rice Vinegar, Safeway Inc., Pleasanton, CA) was used as a drowning solution.[12] For treatment 2, the previously identified W2 blend was composed of acetic acid (1.6%) and ethanol (7.2%) released from the drowning solution and 1.0 mL of a mixture of acetoin (23%), grape butyrate (1%), methionol (2%), isoamyl lactate (1%), 2-phenylethanol (47%) and diethyl succinate (26%) released from a single vial.[13] For treatment 3, the V2 blend was composed of acetic acid (1.6%) and ethanol (7.2%) released from the drowning solution and 1.8 mL of a mixture of acetoin (54%), grape butyrate (7%) and 2-phenylethanol (39%) released from a single vial.[13] For treatment 4, acetoin, ethyl lactate and methionol were released from separate vials each containing 2.0 mL of 50% (w/w) acetoin dissolved in water, 1 mL of ethyl lactate or 1 mL of methionol, and acetic acid and ethanol were formulated in the drowning solution at 1.6 and 7.2% respectively.

2.6 Drop-out study

The objective of this study was to determine whether all of the five compounds (i.e. acetic acid, ethanol, acetoin, ethyl lactate and methionol) tested as a new blend in the preceding experiment were key to the attractiveness of that blend to *D. suzukii*. The study was conducted from 4 to 18 September 2012 in Marion County, Oregon. Mature and overripe blackberries were abundant during the study. The five-component blend of the preceding study was compared with all possible four-component combinations, each of which omitted one of the five chemicals. The hypothesis was that the *D. suzukii* response to each of these four-component blends would be significantly less than the response to the five-component blend, indicating the importance of the chemical omitted.

As in the previous studies, the ethanol and acetic acid were put into the trap drowning solution, and acetoin, ethyl lactate and methionol were released from vials. Treatments were: (1) the new five-component *D. suzukii* blend; (2) the five-component blend minus ethyl lactate; (3) the five-component blend minus acetoin; (4) the five-component blend minus methionol; (5) the five-component blend minus acetic acid; (6) the five-component blend minus ethanol. For treatments that included ethanol and/or acetic acid, drowning solutions (300 mL) included the ethanol at 7.2% and/or the acetic acid at 1.6% with boric acid and soap. Acetoin, ethyl lactate and methionol were released from separate vials each containing 2.0 mL of 50% (w/w) acetoin dissolved in water, 1 mL of ethyl lactate or 1 mL of methionol respectively.

2.7 Statistical analyses

For all studies, a randomized complete block design was used with ten replications. Male and female fly trap catches over 2 weeks were totalled for each replicate and analyzed with block as a random factor and different odor sources as a fixed factor using SAS Proc Mixed (v.9.2).[15] Fly catch data were square root transformed to improve normality and homoscedasticity.[16] For the add-on study, the effects of linear combinations of independent variables were compared using contrast statements in SAS Proc Mixed.[17] Predetermined specific contrasts made were comparisons of 'blank (Trt 1) versus a mixture of acetic acid and ethanol (Trt 4)' to test whether the individual add-on trapping tests were reliable, 'blank (Trt 1) versus an individual compound (Trt 2 or Trt 3)' to test whether individual chemicals could be attractive to *D. suzukii* and 'a mixture of acetic acid (Trt 4) versus a combination of an individual chemical with a mixture of acetic acid and ethanol (Trt 5 or Trt 6)' to determine coattractants with a mixture of acetic acid and ethanol. For the new synthetic blend study and drop-out study, the treatment means were compared using the Tukey–Kramer test in SAS Proc Mixed.[15]

3 RESULTS

3.1 Add-on study

Field trapping experiments showed that 11 out of the 13 EAD-active wine and vinegar chemicals were not attractive to *D. suzukii* when presented by themselves. In most cases, the numbers of *D. suzukii* captured in traps baited with single compounds did not differ from the numbers of *D. suzukii* captured in traps baited with water as the drowning solution (with soap and boric acid). The exceptions were the numbers of male and female *D. suzukii* captured in traps baited with acetoin (contrast 'blank' versus 'acetoin'; $F_{1,45} = 8.73$, $P = 0.005$ for male and $F_{1,45} = 9.11$, $P = 0.004$ for female) or the numbers of male *D. suzukii* captured in traps baited with ethyl lactate (contrast 'blank' versus 'ethyl lactate'; $F_{1,45} = 5.05$, $P = 0.030$) which were greater than the numbers of flies captured in traps baited with water (Figs 1a and b).

The mixture of acetic acid and ethanol was more attractive to *D. suzukii* than the unbaited control (water with soap and boric acid), with two exceptions. The numbers of male and female flies captured in traps baited with the mixture of acetic acid and ethanol were significantly greater (for all contrasts 'water' versus 'acetic acid + ethanol'; $P < 0.046$), except for two cases (Figs 1d and f) where the numbers of male flies captured in traps baited with the mixture of acetic acid and ethanol were not different compared with the numbers captured in traps baited with water. The numbers of flies captured in traps baited with different treatments were significantly different (overall *F*-tests; $P < 0.02$ for male flies and $P < 0.002$ for female flies) for all seven trapping tests of the add-on study (Figs 1a to g), except for male flies captured in one test (Fig. 1f).

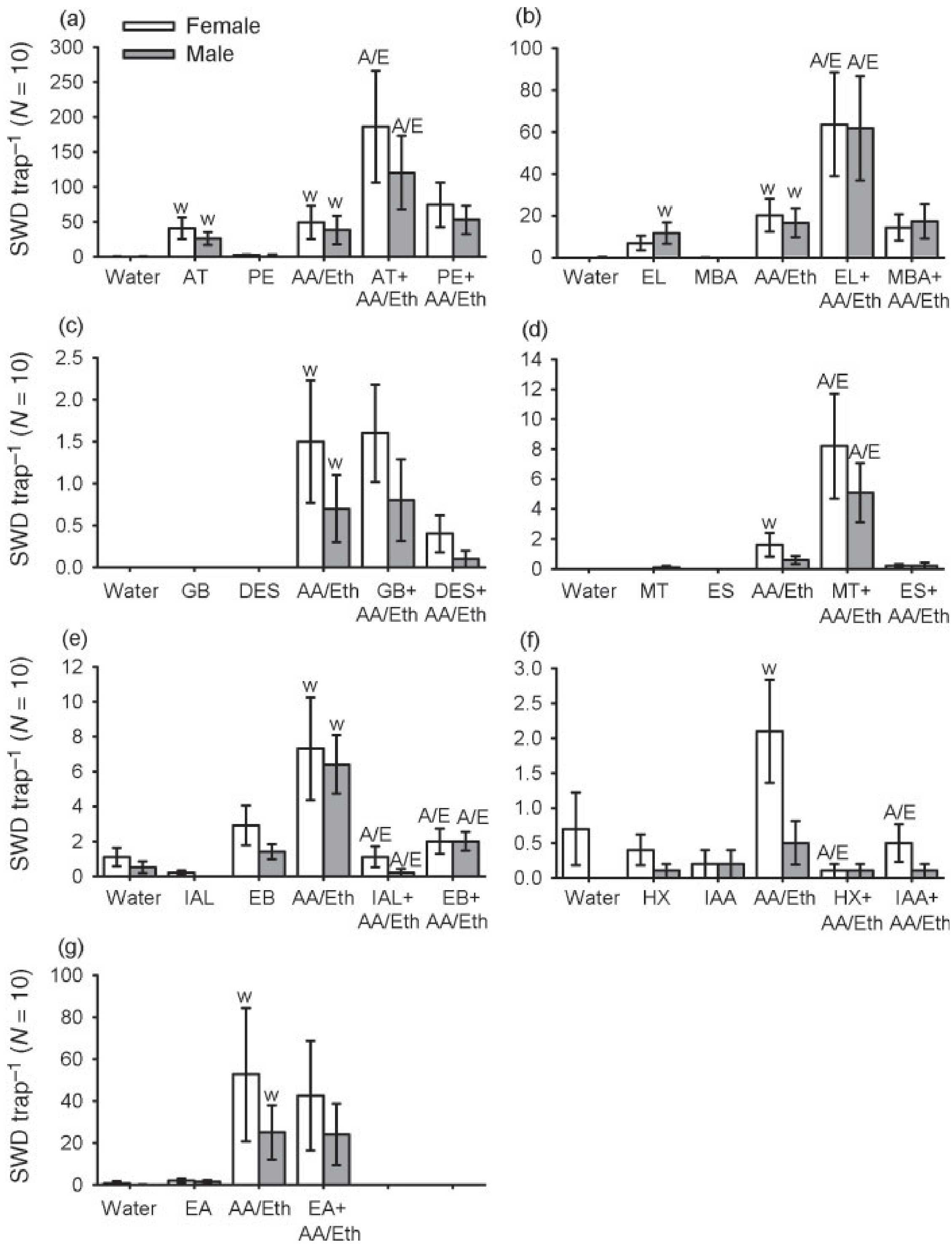

Figure 1. Mean (± SEM) numbers of male and female *Drosophila suzukii* flies captured in traps baited with a control (water), a mixture of acetic acid and ethanol (AA/Eth), one of EAD-active chemical and a combination of the EAD-active chemical and a mixture of acetic acid and ethanol. Up to two EAD-active chemicals were tested in each experiment. The EAD-active chemicals tested were: (a) acetoin (AT) and 2-phenylethanol (PE); (b) ethyl lactate (EL) and 2-methylbutyl acetate (MBA); (c) grape butyrate (GB) and diethyl succinate (DES); (d) methionol (MT) and ethyl sorbate (ES); (e) isoamyl lactate (IAL) and ethyl butyrate (EB); (f) 1-hexanol (HX) and isoamyl acetate (IAA); (g) ethyl acetate (EA). Letter 'w' on bars indicates significant differences with traps baited with water (control) by using contrast statements in SAS Proc Mixed at $P < 0.05$. Letters 'A/E' on bars indicate significant differences with traps baited with AA/Eth by using contrast statements in SAS Proc Mixed at $P < 0.05$. Statistical tests were based on square-root-transformed data. Means from untransformed data are shown. ANOVA: (a) for female, $F_{5,45} = 12.34$, $P < 0.001$; for male, $F_{5,45} = 12.16$, $P < 0.001$; (b) for female, $F_{5,45} = 10.92$, $P < 0.001$; for male, $F_{5,45} = 10.00$, $P < 0.001$; (c) for female, $F_{5,45} = 4.85$, $P = 0.001$; for male, $F_{5,45} = 3.28$, $P = 0.013$; (d) for female, $F_{5,45} = 11.03$, $P < 0.001$; for male, $F_{5,45} = 8.52$, $P < 0.001$; (e) for female, $F_{5,45} = 6.03$, $P < 0.001$; for male, $F_{5,45} = 15.01$, $P < 0.001$; (f) for female, $F_{5,45} = 3.89$, $P = 0.005$; for male, $F_{5,45} = 1.05$, $P = 0.402$; (g) for female, $F_{3,27} = 9.46$, $P < 0.001$; for male, $F_{3,27} = 7.62$, $P < 0.001$.

Table 1. List of seven trapping experiments with treatments and names of EAD-active chemicals tested in each of the seven 'add-on' field tests. Up to two EAD-active compounds were tested in each experiment. EAD-active chemicals were released individually from a 4 mL polypropylene vial with a 3 mm diameter hole in the lid, except for ethyl acetate, which was released as part of the drowning solution as 2% ethyl acetate in H_2O. AA: acetic acid; EtOH: ethanol; EA: ethyl acetate; AT: acetoin; PE: 2-phenylethanol; EL: ethyl lactate; MBA: 2-methylbutyl acetate; GB: grape butyrate; DES: diethyl succinate; MT: methionol; ES: ethyl sorbate; IAL: isoamyl lactate; EB: ethyl butyrate; HX: 1-hexanol; IAA: isoamyl acetate; OR: Oregon; MS: Mississippi[a]

Date	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Site
23/2/12	Blank	AA/EtOH	EA	EA + AA/EtOH			OR
14/3/12	Blank	AA/EtOH	AT	AT + AA/EtOH	PE	PE + AA/EtOH	OR
29/3/12	Blank	AA/EtOH	EL	EL + AA/EtOH	MBA	MBA + AA/EtOH	OR
20/4/12	Blank	AA/EtOH	GB	GB + AA/EtOH	DES	DES + AA/EtOH	OR
20/4/12	Blank	AA/EtOH	MT	MT + AA/EtOH	ES	ES + AA/EtOH	OR
11/6/12	Blank	AA/EtOH	IAL	IAL + AA/EtOH	EB	EB + AA/EtOH	MS
6/7/12	Blank	AA/EtOH	HX	HX + AA/EtOH	IAA	IAA + AA/EtOH	MS

[a] The drowning solution in all treatments contained boric acid and soap. The drowning solution for Trt 1, Trt 3 and Trt 5 was 300 mL of H_2O. One exception was Trt 3, for which the drowning solution was 300 mL of 2% ethyl acetate in H_2O. The drowning solution for Trt 2, Trt 4 and Trt 6 was 300 mL of 7.2% ethanol + 1.6% acetic acid in H_2O. One exception was Trt 4, for which the drowning solution was 300 mL of 2% ethyl acetate + 7.2% ethanol + 1.6% acetic acid in H_2O.

When presented with the mixture of acetic acid and ethanol, acetoin, ethyl lactate or methionol significantly increased the numbers of *D. suzukii* captured compared with the numbers captured in traps baited with the mixture of acetic acid and ethanol (Figs 1a, b and d). Traps baited with the combination of acetoin and the mixture of acetic acid and ethanol captured 3.2 times more males and 3.8 times more females compared with the traps baited with the mixture of acetic acid and ethanol (contrast 'acetic acid + ethanol' versus 'acetoin + acetic acid + ethanol'; $F_{1,45} = 10.42$, $P = 0.002$ for male and $F_{1,45} = 12.46$, $P = 0.001$ for female). Traps baited with the combination of ethyl lactate and the mixture of acetic acid and ethanol captured 3.7 times more males and 3.1 times more females compared with the traps baited with the mixture of acetic acid and ethanol (contrast 'acetic acid + ethanol' versus 'ethyl lactate + acetic acid + ethanol'; $F_{1,45} = 8.05$, $P = 0.007$ for male and $F_{1,45} = 7.54$, $P = 0.009$ for female). Traps baited with the combination of methionol and the mixture of acetic acid and ethanol captured 8.5 times more males and 5.1 times more females compared with the traps baited with the mixture of acetic acid and ethanol (contrast 'acetic acid + ethanol' versus 'methionol + acetic acid + ethanol'; $F_{1,45} = 14.28$, $P = 0.001$ for male and $F_{1,45} = 15.00$, $P < 0.001$ for female). In contrast, when added to the mixture of acetic acid and ethanol, isoamyl lactate and ethyl butyrate significantly reduced the numbers of male and female *D. suzukii* captured (contrast 'acetic acid + ethanol' versus 'isoamyl lactate + acetic acid + ethanol'; $F_{1,45} = 45.79$, $P < 0.001$ for male and $F_{1,45} = 15.59$, $P < 0.001$ for female; contrast 'acetic acid + ethanol' versus 'ethyl butyrate + acetic acid + ethanol'; $F_{1,45} = 9.90$, $P = 0.003$ for male and $F_{1,45} = 8.81$, $P = 0.005$ for female), and 1-hexanol and isoamyl acetate significantly reduced numbers of female *D. suzukii* captured (contrast 'acetic acid + ethanol' versus '1-hexanol + acetic acid + ethanol'; $F_{1,45} = 7.88$, $P = 0.007$; contrast 'acetic acid + ethanol' versus 'isoamyl acetate + acetic acid + ethanol'; $F_{1,45} = 14.88$, $P < 0.001$) (Figs 1e and f).

3.2 New blend study

Numbers of male and female flies captured in traps baited with the new five-component blend of acetic acid, ethanol, acetoin, ethyl lactate and methionol were not different from or were even greater than the numbers of flies captured in traps baited with the mixture of wine plus vinegar (Fig. 2). In Oregon, traps baited with the new five-component blend captured similar numbers of male and female *D. suzukii* flies to traps baited with the mixture of wine and vinegar (Fig. 2a). In Mississippi, traps baited with the five-component blend captured greater numbers of female flies (Tukey–Kramer: $P = 0.047$) and similar numbers of male flies to traps baited with the mixture of wine and vinegar (Fig. 2b). At both locations, the numbers of male and female flies captured in traps baited with previously identified wine- and vinegar-based chemical blends (W2 and V2 respectively) that were based on GC-EAD and laboratory 'add-on' tests[12] were significantly lower compared with the numbers captured in traps baited with the mixture of wine and vinegar (Tukey–Kramer test: for all comparisons $P < 0.001$) or compared with the numbers captured in traps baited with the new five-component blend (Tukey–Kramer test: for all comparisons $P < 0.001$) (Fig. 2). For both trapping tests (Figs 2a and b), the numbers of flies captured in traps baited with different treatments were significantly different [overall *F*-test; $F_{3,27} = 14.92$, $P < 0.001$ for female flies and $F_{3,27} = 20.34$, $P < 0.001$ for male flies in Oregon (Fig. 2a); $F_{3,27} = 40.54$, $P < 0.001$ for female flies and $F_{3,27} = 29.40$, $P < 0.001$ for male flies in Mississippi (Fig. 2b)].

3.3 Drop-out study

The numbers of male and female flies captured in traps baited with the five-component blend or five different four-component subtraction blends were significantly different ($F_{5,44} = 30.05$, $P < 0.001$ for female flies; $F_{5,44} = 30.92$, $P < 0.001$ for male flies) (Fig. 3). Removing acetoin, methionol, acetic acid or ethanol from the five-component blend significantly reduced the numbers of male and female flies trapped compared with the numbers of male and female flies trapped with the five-component blend (male and female combined average decrease by removing acetoin was 61%, for methionol 69%, for acetic acid 95% and for ethanol 80%; for all comparisons, Tukey–Kramer: $P < 0.001$). In contrast, removing ethyl lactate from the five-component blend did not significantly impact upon the numbers of male and female flies captured compared with the numbers of male and female flies captured in traps baited with the five-component blend (Fig. 3).

4 DISCUSSION

A chemical blend composed of four key volatile compounds (acetic acid, ethanol, acetoin and methionol) that is attractive

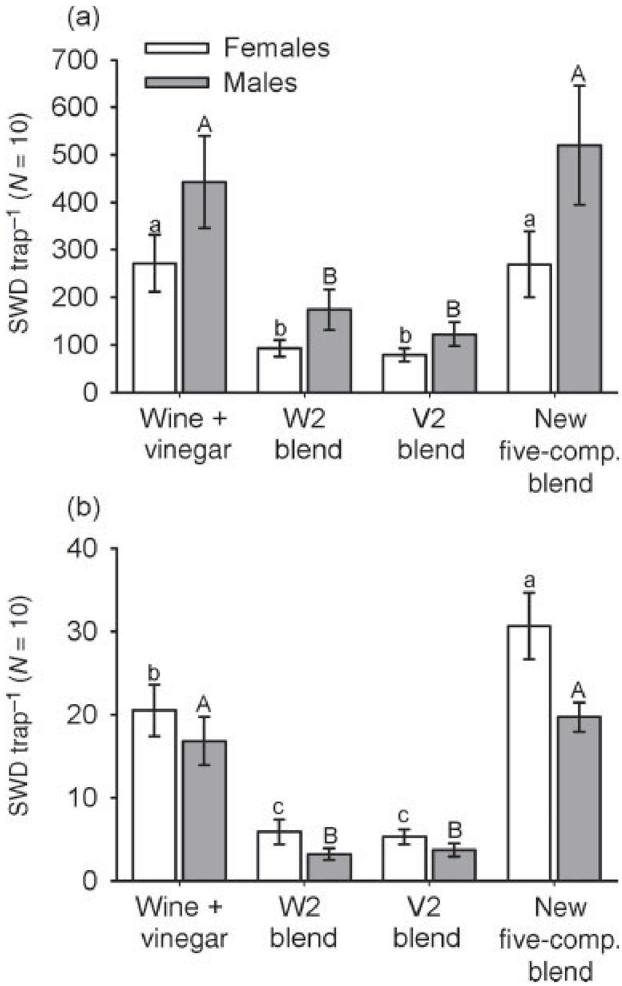

Figure 2. Mean (± SEM) numbers of male and female *Drosophila suzukii* flies captured in traps baited with a mixture of wine and vinegar (W + V), a previously identified wine (W2) blend and vinegar (V2) blend and a new five-component *D. suzukii* blend at (a) the Oregon site and (b) the Mississippi site. For a given sex, different letters on bars indicate significant differences by Tukey–Kramer tests at $P < 0.05$. Statistical tests were based on square-root-transformed data. Means from untransformed data are shown.

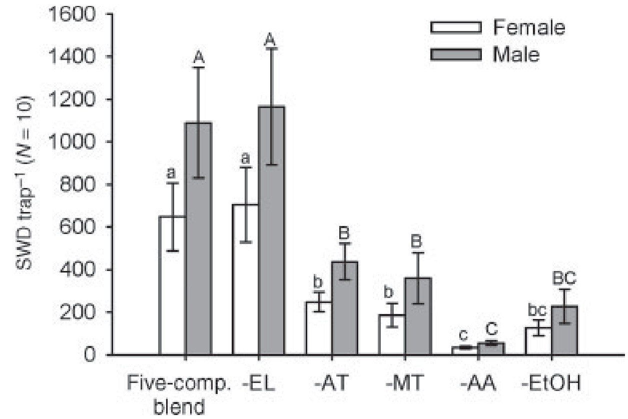

Figure 3. Mean (± SEM) numbers of male and female *Drosophila suzukii* flies captured in traps baited with the new five-component blend (new blend), the five-component blend minus ethyl lactate (− EL), the five-component blend minus acetoin (− AT), the five-component blend minus methionol (− MT), the five-component blend minus acetic acid (− AA) and the five-component blend minus ethanol (− EtOH) at the Oregon site. For a given sex, different letters on bars indicate significant differences by Tukey–Kramer tests at $P < 0.05$. Statistical tests were based on square-root-transformed data. Means from untransformed data are shown.

to both male and female *D. suzukii* in the field has now been identified. Given that this four-component blend was similar in attractiveness to the five-component blend which was comparable with the wine and vinegar mixture (Figs 2 and 3), it is anticipated that this four-component blend can be used to develop a highly attractive chemical lure with potential for use in the detection and management of *D. suzukii*, once work to develop controlled-release dispensers and to optimize lure parameters such as release rate and component ratios has been completed.

D. suzukii are attracted to fermentation food resources, and recent studies have indicated that the combination of wine and vinegar is much more attractive to *D. suzukii* than wine or vinegar alone.[11,12] Volatiles from wine and vinegar contained a multitude of volatile compounds in addition to ethanol and acetic acid respectively, and 13 of these chemicals elicited antennal responses in *D. suzukii*.[13] In the present field experiment it was found that three among the 13 chemicals (acetoin, ethyl lactate and methionol) were coattractive to *D. suzukii* with the mixture of acetic acid and ethanol (Fig. 1). The resultant five-component blend, composed of acetic acid, ethanol, acetoin, ethyl lactate and methionol, attracted numbers of *D. suzukii* similar or greater in magnitude to the numbers captured by the wine and vinegar mixture in the field (Fig. 2). From the five-component blend, ethyl lactate could be further eliminated without losing the attractiveness. However, eliminating any of the other four compounds individually resulted in significant loss of attractiveness to *D. suzukii* (Fig. 3), suggesting that these four compounds are key to the recognition of fermentation food resources that are required for the survival of both reproducing and non-reproducing *D. suzukii* flies. All of these five compounds are produced by various microorganisms such as yeast, acetic acid bacteria and lactic acid bacteria in wines and vinegars.[18–24]

The fact that ethyl lactate could be omitted from the five-component blend without significantly diminishing the lure attractiveness suggests that there may be redundancy in the olfactory recognition of food by *D. suzukii*. Redundancy means that not all components in an attractive blend are essential for recognition of the blend, and that it is possible to substitute certain components with other ones without significantly affecting the odor recognition.[25–27] This flexibility in odor perception and recognition in the insect brain may be an adaptation to inherent variation in volatile profiles emitted from food or host plants of insects, and may explain why different chemical blends based on host- or resource-related chemicals can be similarly attractive.[26,27] In addition, although different types of vinegar and wine have quite different volatile profiles,[28,29] they seem to be collectively recognized by *D. suzukii*,[11] indicating that more than one attractive chemical lure may be possible for *D. suzukii*. Acetoin was coattractive with acetic acid plus ethanol in both the laboratory[13] and the field trapping experiments (Fig. 1a). However, some results of the add-on testing done in the field (Fig. 1) were not consistent with previously reported add-on tests conducted in laboratory bioassays.[13] While both methionol and ethyl lactate were coattractive with the mixture of acetic acid and ethanol in the field experiments (Figs 1b and d), methionol had no significant effect, either positive or negative, in the laboratory study, and ethyl lactate significantly decreased the attractiveness of the acetic acid plus ethanol mixture in the laboratory. Discrepancies in insect response to odor in field experiments versus laboratory bioassays are not uncommon[14] and may be attributed to differences in concentrations of chemicals as a result of evaporation and diffusion, in background odor, in spatial parameters and in insect physiological states between laboratory and field conditions. More importantly, this result confirms that careful consideration must be employed when laboratory-bioassay-based results are applied to predict insect behavior in the field.

Although the present experimental results determined that acetoin, ethyl lactate, and methionol are coattractants, it cannot be concluded that other EAD-active compounds are not also attractive. Although they were not 'coattractants' in the present experiment, the results may differ under different circumstances such as a different release rate, attraction assay design or in combination with other chemicals. This situation is suggested in the case of the addition of ethyl lactate to acetic acid and ethanol, with evidence of a negative effect in the laboratory bioassay but indication of coattractiveness in the field test.

A potential benefit of isolating a chemical lure targeted to a specific insect species from a food-type bait is the possibility of reductions in non-target insects trapped. The numbers and variance of volatile compounds emitted by fermented food baits such as wines and vinegars are relatively great. A synthetic lure comprising a small percentage of these headspace volatiles might be expected to attract a correspondingly smaller range of insects. Therefore, a synthetic lure with a minimum set of chemicals essential for attraction of one species may also minimize the trapping of non-target insects. On the other hand, the capture of non-target insect pests using a chemically complex bait such as a mixture of wine and vinegar provides new opportunities for a better understanding of olfactory chemical attraction and development of chemical lures for these pests. For example, in the second field experiment in Mississippi (Fig. 2b), traps baited with a mixture of wine and vinegar captured 5.2 ($\pm$ 0.87 SE) *Polistes metricus*, 2.6 ($\pm$ 0.58 SE) *P. bellicosus* and 25.6 ($\pm$ 3.58 SE) *Zaprionus indianus* per trap. All three of these species were non-targets in this study but are pestiferous at times,[30,31] with a need for baits and attractants.

Release rates of chemicals from vials and drowning solutions are influenced by various factors, including vapor pressure, temperature, concentration and hole size, and the performance of the trap may be compromised if key chemicals are depleted. Weight loss data of vials (3 mm diameter hole in the lid) containing 2 mL of 50% (w/w) acetoin show that acetoin and water were released at 38 mg day^{-1} over 2 weeks in a laboratory hood (22.5 $\pm$ 1 $^{\circ}$C), suggesting that acetoin could be released at least for 26 days with about 1 g of acetoin loaded into the vial. Methionol is hygroscopic, and accurate gravimetric measurement of the release rate from vials was not possible owing to the potential for adsorption of water from the atmosphere. However, as the vapor pressure of methionol (0.16 mmHg at 25 $^{\circ}$C) is lower than that of acetoin (2.69 mmHg at 25 $^{\circ}$C), methionol with around 1 g loading also should not have been depleted during the 2 week trapping period. On the other hand, it is unclear whether some of the chemicals in the vials containing W2 blends and V2 blends were depleted over the trapping period. In these two blends, methionol and acetoin were loaded at lower doses (0.02 g methionol in the W2 blend and 0.2 and 0.6 g acetoin in the W2 and V2 blends respectively), as part of a mixture of compounds, than the doses used in the new blend of the present study (1 g each). In addition, the release rates of the individual chemicals of such a blend would differ from the release rates of the same chemicals dispensed from separate vials. These two parameters, as well as the variance in release rates with field temperatures (e.g. colder early spring temperature in the previous study versus warmer late summer in the present study), impact also upon the longevity of the lure. Thus, inconsistencies in the relative performance of the W2 and V2 blends (here and in the previous study[13]) may be due to a variety of parameters impacted upon by the selection of methods.

This work was based on *D. suzukii* attraction to man-made fermentation products, a wine and a vinegar, and was not based directly on *D. suzukii* food-finding behavior in a natural environment and natural context. Therefore, the amounts and release rates of materials and chemicals tested were not based on volatile release rate information from a fly food source in nature, and neither were they based on any empirical determination of optimum fly response to release rates of wine and vinegar mix volatiles. Further experimentation is needed, then, to determine the optimum release rates and ratios of these chemicals required to obtain a best response by *D. suzukii* in the field, with no *a priori* expectation that the dispensing systems and chemical release rates used in these experiments are optimum. Additional experimentation may also be needed to determine whether water is an additional component of the bait, and whether additional volatiles from other fermented food materials are also attractive to *D. suzukii*.

As with any insect chemical attractant, the power and effectiveness of the four-component feeding attractant for *D. suzukii* may be impacted upon by the presence or absence of competing resources and resource signals. An abundance of fermenting fruits might reduce the need by flies to seek food, reducing their attraction response to any chemical lure that functions as a feeding attractant. Also, an abundance of fermenting fruits might emit an abundance of volatiles that would compete with a feeding attractant in a trap, or mask the chemicals emitted from a trap lure. It will be important with any such lure to determine how the lure performance varies in the field relative to the phenology of the crop. Of course, these concerns and research needs apply just as well to the food-grade baits, such as apple cider vinegar, that are currently in use.

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